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Scientific Note

# Genetic Structure of Three Populations of *Oxya chinensis* in Shanxi, China

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**Abstract**: Starch gel electrophoresis was used to analyze genetic structure of three populations of *Oxya chinensis* from Shanxi Province, China. Four allozyme polymorphic loci (MDH-1, MDH-2, LDH, ME) were found. The data suggest that two populations of *O. chinensis* about 41 kilometers apart are genetically similar, but differentiated from the third population with a geographic distance of 357 kilometers. However, the heterozygosity levels of those two genetically similar populations were much different. The Nei's genetic distance between two geographically close populations is 0.068, which is much smaller than those of geographically distant populations (0.23 in average). The observed overlap of geographical distance and genetic distance implies that for *O. chinensis* population divergence may correlate with geographic isolation.

**Key words**: *Oxya chinensis*; Allozyme; Population genetics; Shanxi

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# 中华稻蝗三种群遗传结构分析

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**摘要**：应用淀粉凝胶电泳对山西省太原黄陵村、临猗县赵村及永济县伍姓湖3个中华稻蝗（*Oxya chinensis*）种群的4个等位酶位点（MDH-1、MDH-2、LDH、ME）进行了分析。结果表明3个种群的中华稻蝗在遗传结构上具有差异：黄陵种群的MDH-1、MDH-2以及伍姓湖种群的MDH-1均为单态位点；赵村种群的杂合性最低（$H=0.112$），伍姓湖种群最高（$H=0.229$）；赵村种群与伍姓湖种群间的Nei's遗传距离为0.068，可视为1个大的种群，而黄陵种群与赵村种群和伍姓湖种群的Nei's遗传距离分别为0.247和0.218。考虑到赵村和伍姓湖之间的地理距离（41 km）比太原黄陵（357 km）要近得多，结果提示3个种群地理距离与遗传结构差异之间存在相关关系。

**关键词**：中华稻蝗；等位酶；种群遗传；山西省

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*Oxya chinensis* (Orthoptera, Acrididea, Catantopidae, *Oxya* Serville) represents an important agricultural pest to rice and other crops. This grasshopper species widely and abundantly distributes in most part of China and the adjacent regions. Taxonomically *O. chinensis* was previously described as a species complex with several species involved (Ma, 1991). However, due to the subtlety of morphological characteristics used for species definition, some researchers suggested that as many as ten described species could be grouped as one species of *Oxya chinensis* (Hollis, 1971, 1975). In the past ten years the comparative studies on chromosome have showed that the C-band types of different *O. chinensis* populations across China (from Heilongjiang to Fujian Province) demonstrated very high similarity (Ma *et al*., 1994), indicating the relatively low differentiation among *O. chinensis* populations. More recent research on the cytochrome-*b* gene in mitochondrial DNA also supports the view that the *O. chinensis* populations are less divergent than those of other grasshopper species (Wang, 2001).

However, several studies have noted quantitative morphological difference among *O. chinensis* populations. For example, there exists an increasing cline of body size of *O. chinensis* from the north to south in China. The body length of the samples from Nenjiang, Heilongjiang Province (22.45 ± 2.05 mm) is much smaller than those from Fuzhou, Fujian Province (33.65 ± 2.45 mm) (Guo *et al*., 2001). Another research examined other morphological characters between two populations from Chang'an and Hanzhong, Shaanxi Province. The data suggest that the quantitative differences are significant enough to define *O. chinensis* from those two sites as relatively independent different demes (Wang *et al*., 1997). Because the body size and other quantity characters of *O. chinensis* are genetically determined and stable among the populations, it becomes necessary to examine the genetic diferentiation of *O. chinensis* at molecular level in order to explore the possible evolutionary history of this species.

Allozyme analysis has been used as a valuable method to examine population genetic structure and compare differentiations. It has been successfully employed to study a wide variety of taxa. However, its application to grasshopper is scarce. Zheng *et al*. (1986) compared esterase zymograms in 8 locust species from China. Later the study was expanded by using two more isozymes of malate dehydrogenase and lactate dehydrogenase on 67 species with a few individuals per species (Huang, 1990) The present study used allozyme analysis to examine the genetic structure of three populations of *O. chinensis* from Shanxi Province in Midwest China.

The hypothesis to be tested is that the geographically isolated populations of *O. chinensis* may demonstrate genetic differentiation at allozyme level and the divergence levels may correlate to geographic distance.

## 1 Methods and Materials

### 1.1 Sample collection

Three populations of *O. chinensis* were all collected from Shanxi Province, China. They are from Huangling in suburb of Taiyuan (E 112°36′, N 37°46′), Zhaocun Villege, Linyi County (E 110°24′, N 35°15′), and Wuxinghu, Yongji County (E 110°28′, N 34°53′), in July and August, 2000.

Huangling , Zhaocun and Wuxinghu collection areas are a grassplot, a beach with some ruderal of the grass family, and a thick reed at Wuxinghu, respectively.

The insect samples were brought to laboratory alive and then frozen at −20℃ until electrophoresis. No observable mortality occurred during the transportation.

### 1.2 Methods

Starch gel was prepared using a mixture of 12.5 g soluble starch, 6.25 g potato starch for electrophoresis (Sigma) and 6.25 g refined potato starch (prepared in our laboratory) per 200 mL of buffer. The final starch gel concentration is 12.5% (w/v). Tris-citric acid (0.1 mol/L, pH8.0) was used as electrode buffer. The ratio of electrode buffer to gel buffer is 9∶1. Gel was prepared in a mold of 135 mm × 135 mm × 7 mm. The cooled gel was then stored at 4℃for 2 hours before used.

The insect tissue was from femur muscle. The tissue was removed and then homogenated in 20 μL double distilled water on an ice pan. Filter paper was cut into wicks for loading sample. Electrophoresis lasted for 4 hours at 4℃, at a voltage of 140 V. The initial current was around 25 mA. Allozyme staining was conducted following the standard method (Murphy *et al*, 1996) with modifications. The band with the fastest migration is designated "A", the second "B", and so on. Three allozymes, which were lactate dehydroge nase ( LDH ) , malate dehygrogenase ( MDH ) and malic enzyme ( ME ), were used to examine. The LDH substrate was prepared using lactic acid (Wuhan Dongshan Lactic Acid Fatory) and adjusting pH with NaOH to a final concentration of 1 mol/L. Several days later, the pH of this sodium lactate solution dropped to as low as 6.5. It has to be readjusted back to 8 before staining.

### 1.3 Statistics

BIOSYS-Ⅱ was used to calculate allele frequency, percent polymorphic loci, heterozygosity ($H$), goodness-of-fit to Hardy-Weinberg's (H-W) equilibrium, Nei's genetic identity ($I$) and Roger's genetic distances ($D$) (Nei, 1987). The genetic distance was clustered with unweighted pair-group method using arithmetic averages (UPGMA).

## 2 Results

### 2.1 Locus

Using the methods in this study, all three allozymes were successfully stained with sufficient separation and clear bands that could be scored with minimum efforts. MDH has shown two loci, one (designed as MDH-1) migrated from cathode to anode, and the other (MDH-2) moved from anode to cathode. MDH-1 has three subbands. LDH and ME each have one locus, moving from cathode to anode.

### 2.2 Allele

Table 1 presents the allele frequency of polymorphic loci for each population.

### 2.3 Heterozygosity

In three populations, heterozygosity of Zhaocun is the lowest ($H = 0.112$) and that of Wuxinghu is the highest ($H = 0.229$) (Table 2).

### 2.4 H-W equilibrium

The deviation from H-W equilibrium was observed in the following loci: ME of Huangling population; all loci of Zhaocun population; and MDH-2 and ME of Wuxinghu population. (Chi-square test, $P < 0.01$) (Table 3).

### 2.5 Nei's genetic identity and Roger's genetic distance

Nei's genetic identity and Roger's genetic distance (Table 4) showed that Zhaocun population and Wuxinghu population were genetically identical, so they can be regarded as two parts of a whole population. However, the genetic identities between these two populations and Huangling population are greater ($D = 0.83 - 0.85$), a typical value for populations in a single species. Cluster based on Roger's genetic distance is presented in Fig.1, and the increase in genetic distance along with geographical distance is shown in Fig.2.

## 3 Discussion

Several alleles of all the three populations of *O. chinensis* have deviated from H-W equilibrium. In most cases, the deviation is because of heterozygote deficiency. Due to species' relatively low flight capability and the water requirement for habitats, without excluding the possible inbreeding and null alleles, population subdivision and selection may be attributed to such deviation (Duan *et al*., 1997, 2000). For example, the Nei's genetic identity between Zhaocun and Wuxinghu populations is as high as 0.988, suggesting that they are from the same population. However, population subdivision is indicated by their dif-

**Table 1 Allele frequency of polymorphic loci among three populations of *Oxya chinensis* in Shanxi**

| Locus | Population | | |
|---|---|---|---|
| | Huangling | Zhaocun | Wuxinghu |
| MDH-1 | | | |
| *N* | 53 | 25 | 24 |
| A | 0.019 | 0.160 | 0.000 |
| B | 0.953 | 0.820 | 0.958 |
| C | 0.028 | 0.020 | 0.042 |
| MDH-2 | | | |
| *N* | 53 | 25 | 24 |
| A | 0.000 | 0.640 | 0.583 |
| B | 0.991 | 0.360 | 0.396 |
| C | 0.009 | 0.000 | 0.021 |
| LDH | | | |
| *N* | 50 | 26 | 26 |
| A | 0.010 | 0.000 | 0.000 |
| B | 0.750 | 0.808 | 0.808 |
| C | 0.240 | 0.192 | 0.192 |
| ME | | | |
| *N* | 37 | 28 | 27 |
| A | 0.216 | 0.214 | 0.278 |
| B | 0.608 | 0.446 | 0.370 |
| C | 0.176 | 0.339 | 0.352 |

ferences in heterozygosity and percentage of polymorphic loci (Table 2). Moreover, all the four loci in Zhaocun are deviated from H-W equilibrium, but such deviation occurs at two loci in Wuxinghu population. Therefore, it is likely that the Zhaocun's samples of *O. chinensis* is a subdivision of Wuxinghu population. Besides, the parthenogenesis has been observed in *Oxya formosana*, a sibling species of *O. chinensis* (Hong & Ando, 1998). Parthenogenesis can make the population deviate from H-W equilibrium. It is not clear whether there exists parthenogenesis in *O. chinensis*.

Chromosome analysis of *O. chinensis* indicates C-band type has an extremely high similarity among *O. chinensis* populations (Ma *et al.*, 1994). The allozyme data, however, suggests quantitative differentiation. The different perspectives of the two methods may have contributed to this observed variance. In karyotic analysis C-band indicates heterochromatin section on chromosome constituting highly repeated DNA sequence that shows the least biological activity, and therefore is conservative (Ma *et al.*, 2000). Allozyme genes, however, represent the most active genes in organisms and are subject to founder effect and natural selection. It implies that for *O. chinensis* the differentiation showed by allozyme level may not be revealed by chromosome analysis.

The three populations of *O. chinensis* sampled in this study were from Shanxi Province with a geographic distance from 41.4 to 372 km. Noticeably, a larger genetic distance between two populations was observed as the geographic distance increased (Fig 2). It suggests a positive correlation between those two important measurements in population genetics meaning that distance-by-isolation plays an important role in population's divergence. Even so more studies are needed to reconfirm this observation with expanded sampling areas and increased number of allozymes, our results offer some implications for the relationship between geographic barrier and population genetic variation in *O. chinensis*.

The life cycle of *O. chinensis* depends on much water and the species' dispersal capacity is relatively limited in terms of flight ability when compared with some other insects of Orthoptera like *Locusta migratoria*, therefore it is reasoned that a moderate geographical

**Table 2 Genetic variability of three populations of *Oxya chinensis* in Shanxi**

| Population | Mean sample size per locus | Mean No. of alleles per locus | Percentage of polymorphic loci* | Mean heterozygosity | |
|---|---|---|---|---|---|
| | | | | Direct-count | H-W expected** |
| Huangling | 48.3±3.8 | 2.8±0.3 | 50.0 | 0.181±0.075 | 0.264±0.126 |
| Zhaocun | 26.0±0.7 | 2.5±0.3 | 100.0 | 0.112±0.052 | 0.437±0.081 |
| Wuxinghu | 25.3±0.8 | 2.5±0.3 | 75.0 | 0.229±0.103 | 0.396±0.108 |

* A locus is considered polymorphy if the frequency of the most common allele does not exceed 0.95; ** Unbiased estimate (Nei, 1987).

**Table 3 Chi-square test for deviation from H-W equilibrium in three populations of *Oxya chinensis* in Shanxi**

| | MDH-1 | MDH-2 | LDH | ME |
|---|---|---|---|---|
| Huangling | 0.103 | — | 1.142 | 19.318* |
| Zhaocun | 14.115* | 26.312* | 16.312* | 24.744* |
| Wuxinghu | 0.022 | 25.107* | 0.023 | 12.026* |

* $P < 0.01$

**Table 4 The genetic similarity and distance in three populations of *Oxya chinensis* in Shanxi**

| | Huangling | Zhaocun | Wuxinghu |
|---|---|---|---|
| Huangling | — | 0.247 | 0.218 |
| Zhaocun | 0.835 | — | 0.068 |
| Wuxinghu | 0.857 | 0.988 | — |

Below diagonal is Nei's genetic identity and above diagonal is Roger's genetic distance.

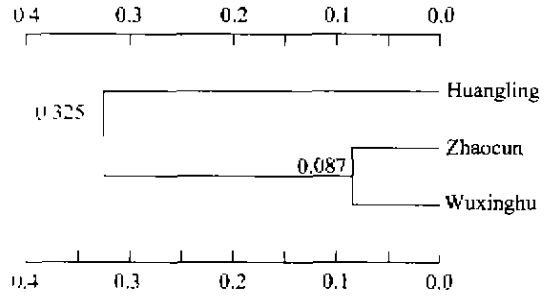


Fig. 1 Cluster analysis of based on Roger's genetic distance in three populations of *Oxya chinensis* in Shanxi (Cophenetic coefficient = 0.997)

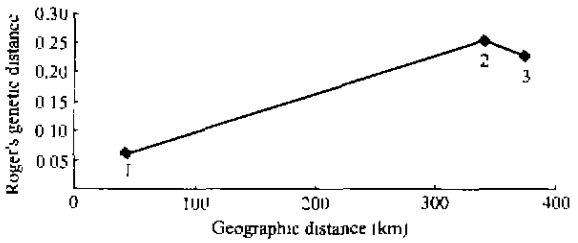


Fig. 2 Relationship between genetic distance and geographic distance in three populations of *Oxya chinensis* in Shanxi

1: the distance between Zhaocun and Wuxinghu; 2: distance between Huangling and Zhaocun; 3. distance between Huangling and Wuxinghu.

barrier may significantly impede the gene flow among *O. chinensis* populations, and high genetic differentiation between the populations of *O. chinensis* may result from distance-by-isolation for a long time, which quantitatively supports our hypothesis that the geographically isolated populations of *O. chinensis* demonstrate genetic differentiation at allozyme level and the divergence levels may correlate to geographic distance.

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